RNA (2002), *8*:1093–1094. Cambridge University Press. Printed in the USA.

DOI: 10.1017.S1355838202027061

LETTER TO THE EDITOR

Problems with the transorientation hypothesis

SCOTT M. STAGG,[1] MIKEL VALLE,[2] RAJENDRA K. AGRAWAL,[3,4] JOACHIM FRANK,[2,3] and STEPHEN C. HARVEY[1]

[1]Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL 35294-0005, USA
[2]Howard Hughes Medical Institute, Health Research Inc., Wadsworth Center, New York State Department of Health Empire State Plaza, Albany, NY 12201-0509, USA
[3]Department of Biomedical Sciences, State University of New York at Albany, Albany, New York 12203, USA
[4]Wadsworth Center, New York State Department of Health, Empire State Plaza, Albany, New York 12201-0509, USA

A novel mechanism for the very first step in polypeptide elongation on the ribosome has recently been proposed by Simonson and Lake (2002). According to their transorientation hypothesis, the incoming aminoacyl-transfer RNA (aa-tRNA) initially binds to the messenger RNA (mRNA) with the anticodon stacked on the 5′ side of the anticodon loop; then, following GTP hydrolysis, the tRNA swings into the A-site by switching from the 5′-stacked conformation to the classic 3′-stacked geometry while maintaining Watson–Crick base pairing with the mRNA. There are several serious problems with this proposal.

Simonson and Lake inferred the position of the aa-tRNA from immunoelectron microscopy mapping of the EF-Tu•GTP•tRNA ternary complex on the 30S ribosomal subunit. Although this procedure may provide a general location for EF-Tu, it is intrinsically incapable of revealing the orientation of the incoming tRNA when the anticodon first approaches the codon in the 70S ribosome. Moreover, the binding of the ternary complex to the isolated 30S subunit at the A-site in the absence of the 50S subunit does not occur as part of a physiological process. There is, at present, no direct experimental evidence supporting the proposed orientation of the EF-Tu•tRNA complex at the putative D-site in the intact ribosome.

One major difficulty with the transorientation model is that it places EF-Tu domain I, which contains the GTPase center, more than 90 Å from the α-sarcin-ricin loop (SRL) of the 50S subunit (Fig. 1). The EF-Tu•GTP•tRNA ternary complex and EF-G are known to have common binding sites on the ribosome, as has been shown by biochemical experiments (Moazed et al., 1988) and by cryo-EM studies (Agrawal et al., 2000a, 2000b). These studies have indicated that the GTP-binding domains of both factors lie within 5 Å of the SRL of the 50S subunit. Because the structures of EF-Tu in the cryo-EM study of the kirromycin-stalled 70S ribosome•EF-Tu•GDP•aa-tRNA complex (Agrawal et al., 2000a) and the one solved by X-ray crystallography of the EF-Tu•GMPPNP•aa-tRNA complex (Nissen et al., 1995) were found to be very similar, and given the fact that kirromycin prevents conformational changes of EF-Tu immediately after GTP hydrolysis, a large conformational change between the GTP and GDP states of the kirromycin-stalled EF-Tu is not expected. The transorientation hypothesis puts EF-Tu in a position where its GTP-binding domain cannot make any contact with the SRL (Fig. 1), even when a very large conformational change in EF-Tu is allowed to take place.

The transorientation model presents serious steric problems as well. The authors acknowledge that there are steric conflicts between EF-Tu and the 50S subunit when EF-Tu is docked at the putative D-site. The transorientation hypothesis requires L11 and nt 1057–1081 of the 23S rRNA to undergo drastic conformational changes to permit binding of the ternary complex. A far simpler explanation is that EF-Tu binds to the ribosome in the same orientation as seen in cryo-EM reconstructions (Fig. 1). In this orientation, EF-Tu can make contact with both L11 and the SRL with no steric conflicts.

In transorientation, a steric clash would also occur when the aa-tRNA swings from the D-site to the A-site, as it would be forced to sweep through parts of the 50S subunit. In the absence of detailed models for possible conformational changes in the 50S subunit, it is not possible to evaluate the claim that transorientation "occurs without significant clash … between the EF-Tu-

Reprint requests to: Stephen C. Harvey, Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL 35294-0005, USA; e-mail: harvey@uab.edu.

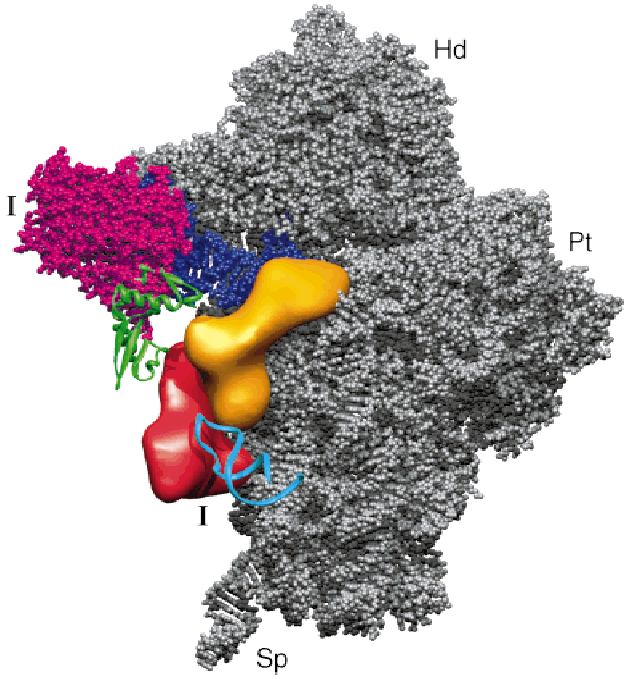

FIGURE 1. Comparison of the positions of the EF-Tu(magenta)•tRNA(blue)•GTP ternary complex from the transorientation model (Simonson & Lake, 2002) with that of the EF-Tu(red)•tRNA(orange)•GDP ternary complex from cryo-EM (Valle et al., 2002). Coordinates for the 30S ribosomal subunit (gray) in the transorientation model (Protein Data Bank accession number 1L1U) were superposed on the 70S crystal structure (1GIX and 1GIY, Yusupov et al., 2001), which was docked into the cryo-EM density map. The position of protein L11 from the large ribosomal subunit is indicated by green ribbons, and the position of the SRL is indicated by the cyan ribbon. The position of domain I of EF-Tu is indicated by an I in both the transorientation model and the cryo-EM reconstruction. Landmarks on the 30S subunit are as follows: Hd: head; Pt: platform; Sp: spur.

aa-tRNA complex and the 70S ribosome." The same difficulty arises when one considers a possible tRNA transition from the proposed D-site to the site derived experimentally by cryo-EM, because steric conflicts with the 50S subunit would also prevent this motion.

The transorientation hypothesis also violates currently accepted principles of tRNA structure. In all high resolution structures of tRNAs to date, the anticodon is stacked on the 3′ side of the loop. It is ironic that Simonson and Lake credit Fuller and Hodgson (1967) for originally proposing the 5′-stacked conformation, as Fuller and Hodgson considered, then rejected the 5′-stacked geometry. Instead, Fuller and Hodgson argued that the anticodons of both A- and P-site tRNAs on the 70S ribosome would be 3′-stacked, and that this would be facilitated by the universal pyrimidine (Y33) at the bottom of the anticodon loop. They then suggested that side-by-side stacking of two 3′-stacked anticodons on the message would require kinking of the mRNA between the A- and P-sites, which is now confirmed in a recent X-ray crystallographic study of the mRNA- and tRNA-bound 70S ribosome (Yusupov et al., 2001).

Finally, it is worth mentioning that the suggestion of 5′-stacking contained in Woese's reciprocating ratchet model (Woese, 1970) was not an unreasonable alternative to the Fuller–Hodgson model at the time it was proposed. Woese argued that kinking the mRNA would be energetically unfavorable, and that the A- and P-site tRNAs would pair most easily with their respective codons on an unkinked mRNA if the A-site anticodon loop were 5′-stacked. He suggested that the switch from 5′ to 3′ stacking would accompany tRNA movement from the A-site to the P-site. Because the global structure of tRNA had not yet been determined, he could not have known that his model would place the two aminoacyl-acceptor termini of the tRNAs about 100 Å apart, precluding peptidyl transfer. Once the tRNA crystal structure was determined, 5′ stacking became untenable and, in the absence of direct experimental evidence to the contrary, it remains so today.

ACKNOWLEDGMENTS

Supported by grants from the National Institutes of Health to S.C.H. (GM53827), R.K.A (GM61576), and J.F. (R37 GM29169)

Received June 6, 2002; accepted without revision June 19, 2002

REFERENCES

Agrawal RK, Heagle AB, Frank J. 2000a. Studies of elongation factor G-dependent tRNA translocation by three-dimensional cryo-electron microscopy. In: Garrett RA, Liljas SRDA, Matheson AT, Moore PB, Noller HF, eds. *The ribosome: Structure, function, antibiotics, and cellular interactions*. Washington: ASM Press. pp 53–62.

Agrawal RK, Spahn CM, Penczek P, Grassucci RA, Nierhaus KH, Frank J. 2000b. Visualization of tRNA movements on the *Escherichia coli* 70S ribosome during the elongation cycle. *J Cell Biol 150*:447–460.

Fuller W, Hodgson A. 1967. Conformation of the anticodon loop in tRNA. *Nature 215*:817–821.

Moazed D, Robertson JM, Noller HF. 1988. Interaction of elongation factors EF-G and EF-Tu with a conserved loop in 23S RNA. *Nature 334*:362–364.

Nissen P, Kjeldgaard M, Thirup S, Polekhina G, Reshetnikova L, Clark BF, Nyborg J. 1995. Crystal structure of the ternary complex of Phe-tRNA^{Phe}, EF-Tu, and a GTP analog. *Science 270*:1464–1472.

Simonson AB, Lake JA. 2002. The transorientation hypothesis for codon recognition during protein synthesis. *Nature 416*:281–285.

Valle M, Sengupta J, Swami NK, Grassucci RA, Burkhardt N, Nierhaus KH, Agrawal RK, Frank J. 2002. Cryo-EM reveals an active role for aminoacyl-tRNA in the accommodation process. *EMBO J 21*:3557–3567.

Woese C. 1970. Molecular mechanics of translation: A reciprocating ratchet mechanism. *Nature 226*:817–820.

Yusupov MM, Yusupova GZ, Baucom A, Lieberman K, Earnest TN, Cate JH, Noller HF. 2001. Crystal structure of the ribosome at 5.5 Å resolution. *Science 292*:883–896.

https://doi.org/10.1017/S1355838202027061 Published online by Cambridge University Press